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THE AXIAL GRADIENTS IN HYDROZOA.

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II. SUSCEPTIBILITY IN RELATION TO PHYSIOLOGICAL AXES, REGIONS OF COLONY AND STAGES OF DEVELOPMENT IN CERTAIN HYDROIDS.

The present paper is a study of the susceptibility relations in certain species of colonial hydroids and includes data bearing upon the following points: axial susceptibility in single hydranths, medusa buds and stems; susceptibility in relation to stage of development, physiological age and motility; regional differences in susceptibility in colonies and their relation to growth form and general physiological condition; susceptibility in acclimation. These data have been accumulated during some five years past, in part at Woods Hole and in part at Friday Harbor, Wash. The forms chiefly studied are *Pennaria tiarella* and *Tubularia crocea* at Woods Hole and *Bougainvillea* sp., *Obelia geniculata* and *O. borealis* at Friday Harbor. Some work was also done with a third unidentified species of *Obelia* and with another campanularian, *Gonothyria clarkii*, also at Friday Harbor. I am indebted to Professor Trevor Kincaid and to Professor C. C. Nutting for identification of certain of the Friday Harbor species.

For the direct determination of susceptibility to slowly lethal concentrations of agents the method is the same as that used for *Hydra* (Child and Hyman, '19) and many other forms, viz., the determination of the time of death as indicated by the disintegration or cytolysis of the protoplasm in KNC, HCl, various anesthetics, neutral red, methylene blue, or in some cases by the time before decoloration in KNC or other neutral or alkaline agents after staining with neutral red. This decoloration occurs only when the protoplasmic structure disintegrates: the first stage is a change in color of the neutral red to yellow as the sea water or the alkaline agent penetrates all parts of the protoplasm,

and following this the yellow color gradually disappears in most cases, the dye being no longer held by the dead protoplasm. The vital dyes, neutral red and methylene blue have been used both as agents for staining *intra vitam* and for determining differences in susceptibility. In the investigation of acclimation in lower concentrations of KCN, HCl, ethyl urethane, MgSO_4 , neutral red and methylene blue, it was found that different hydranths or regions of the colony show differences in capacity for acclimation.

The question of the value of susceptibility as a criterion of certain quantitative aspects of physiological condition has been discussed so many times in earlier papers (see, for example, Child, '19b, and the references there given), that no further consideration of the matter is necessary here. It need only be said that a rapidly increasing volume of evidence along many different lines indicates that in general susceptibility to higher concentrations or intensities of at least many agents, as indicated by death or inhibition, as well as capacity for acclimation to, or recovery from the action of lower concentrations or intensities, vary in general, though not necessarily proportionally to the rate at which fundamental physico-chemical processes are going on in protoplasm.

Since adequate presentation of the experimental protocols on susceptibility would require much space, only the general results are stated, but for each statement made there is an extensive experimental basis. Moreover, the experiments demand no difficult technique and can easily be repeated, the chief precaution to be observed being the distinction between freshly collected material in good condition and that which has been kept in the laboratory. With some species even a few hours under laboratory conditions are sufficient to alter the susceptibility relations of different parts to a considerable degree.

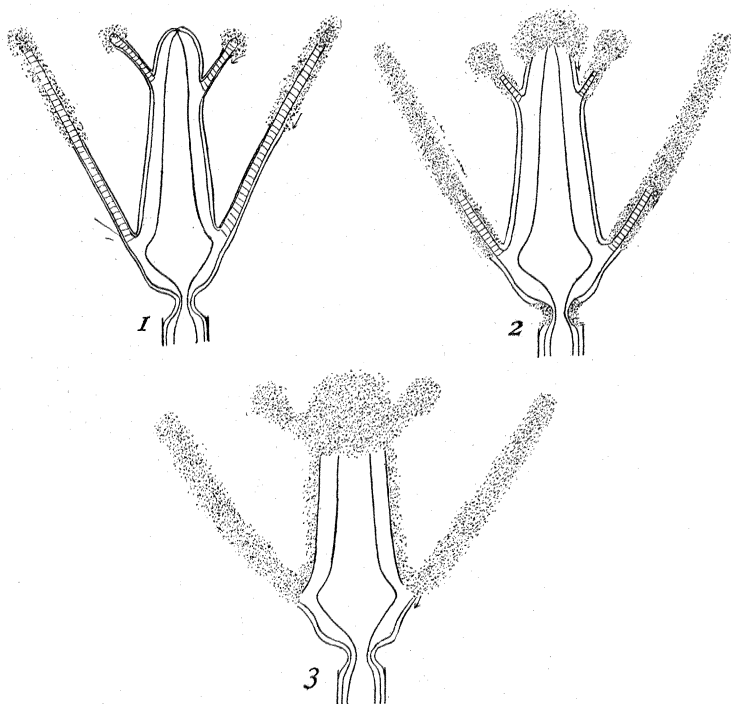
THE DEATH GRADIENTS IN HYDRANTHS.

The observations on hydranths have been made repeatedly, both on the different hydranths of single colonies and different colonies of the species mentioned above, and the progress of disintegration has been observed in hundreds of cases. The

hydranth of *Pennaria tiarella* will serve as an example of the course of disintegration in the hydranth of a tubularian hydroid. In full grown hydranths in KNC $m/800$ to $m/400$ approximately, in neutral red, or in KNC after staining with neutral red to make the death changes more clearly visible, disintegration begins in the ectoderm at the tips of the proximal tentacles and progresses basipetally, usually at about the same rate in each tentacle (Fig. 1). Some time later, usually when disintegration has progressed over one to two thirds the length of the proximal tentacles, it begins in the ectoderm at the tips of the distal tentacles and progresses basipetally in these also, reaching the bases of both series of tentacles at about the same time. At, or often somewhat before this time, ectodermal disintegration begins at the tip of the manubrium and progresses basipetally over the manubrium and body of the hydranth (Figs. 2 and 3). The short stalk below the hydranth differs in relative susceptibility in different cases, and this difference, as in the case of the 'stalk' of *Hydra* (Child and Hyman, '19) apparently depends on the occurrence or non-occurrence of contraction. In cyanide, particularly the higher concentrations, this stalk usually contracts and in such cases its ectoderm disintegrates at about the same time as that of the mouth region (Fig. 2), but where contraction does not occur, it disintegrates only after ectodermal disintegration of the body is completed (Fig. 3).

In general the entoderm disintegrates later than the ectoderm, except near the mouth region. Since the agent must pass through the ectoderm to reach the entoderm, this difference is perhaps not significant, though it may be noted that in neutral red the entoderm stains almost as rapidly as the ectoderm and may stain much more deeply than the latter before death occurs. In the proximal tentacles the solid column of entodermal cells shows a basipetal susceptibility gradient, like the ectoderm, disintegration beginning at the tip of the entodermal column when ectodermal disintegration has progressed half way more or less to the base (Fig. 1). In the distal tentacles the difference in susceptibility between ectoderm and entoderm is similar. In the mouth region the entoderm may disintegrate as early as, or sometimes earlier than the ectoderm, perhaps because the

agent enters through the mouth to some extent, though, so far as observed, the mouth remains closed until disintegration begins in that region. As regards the rest of the body and the stalk, entodermal disintegration is distinctly later than ectodermal and is usually without any very clearly marked gradient.



FIGS. 1-3. Diagrammatic optical sections of *Pennaria* hydranths to show the course of disintegration in lethal concentrations. Fig. 1. Ectoderm of proximal tentacles disintegrated half way to base; entoderm beginning at tips. Ectoderm of terminal knobs of distal tentacles disintegrated. Fig. 2. Ectoderm disintegrated to bases of both series of tentacles; entoderm of proximal tentacles two thirds disintegrated, of distal tentacles half disintegrated. Ectoderm of short stalk below hydranth disintegrating. Fig. 3. Tentacles, hypostome and apical one third of hydranth body completely disintegrated. Ectoderm of hydranth body disintegrated to bases of proximal tentacles. Ectoderm of stalk intact.

Here as in *Hydra* (Child and Hyman, '19, p. 193) the entoderm of the mouth region is apparently much more susceptible than that of other body regions, a difference which is perhaps associated with specially intense glandular activity in this region, of which there is some histological evidence.

The length of time between exposure to the agent and the beginning of disintegration and the rate of its progress may of course be varied widely by varying the concentration. The concentrations most used are those in which disintegration begins in one half to one hour after exposure and is completed in three to six hours.

The susceptibility relations in the well developed hydranth of *Tubularia crocea* and in *Bougainvillea*, except that in the latter there is only one series of tentacles, are similar to those in *Penaria*. The campanularian hydranths examined also show essentially the same relations in fresh material.

THE DEATH GRADIENTS IN STEMS AND STOLONS.

Data on susceptibility of stems to chemical agents, as measured by disintegrative changes, are of course open, in all hydroids possessing a perisarc, to the possible objection that differences in permeability and thickness of the perisarc at different levels may constitute complicating, or perhaps the chief factors in determining the time of death. The thickness of the perisarc is least in the most recently developed, *i. e.*, in any particular axis, the most apical portions of the stem and its thickness increases in general basipetally.

As a matter of fact, however, the perisarc is apparently penetrated rather readily by many agents. It does not obstruct or retard greatly the entrance of vital dyes and permanganate penetrates it almost at once, even where it is thick. While the possible complicating effect of the perisarc must of course be admitted, various facts cited below indicate clearly enough that it is not responsible for all the differences in susceptibility observed in stems.

In general a stem is less susceptible than the hydranth it bears, and the susceptibility decreases basipetally in the stem, at least near the hydranth, in all agents used in lethal concentration. In concentrations of KNC, neutral red, etc., which do not kill within a few hours, the more apical portions of stems usually undergo resorption and retraction within the perisarc after more or less complete disintegration or resorption of the hydranths. These processes of resorption and retraction of the more apical

regions are themselves indications of a differential susceptibility in the stems.

The staining with neutral red may be apparently uniform over many millimeters of stem length, while considerable differences in susceptibility, as indicated by the progress of disintegration and decoloration may appear in the same region. As regards the permeability of the perisarc in different regions to KNC, nothing definite can be said, but the susceptibility gradients in KNC are similar to those in neutral red.

The differences in susceptibility are most marked near the apical ends of stems and stolons, where the differences in rate of growth are greatest within short distances. The growing tip of a stem or a stolon is its most susceptible region, and the susceptibility decreases rapidly from the tip basipetally within a length of several millimeters to a few centimeters, according to the species and the conditions of the stem. In the older regions of stems and stolons differences in susceptibility are much less marked, even over length of several centimeters, and irregularities frequently appear, as in the older portions of the thalli of algæ (Child, '16*a*, '16*b*), some regions dying earlier than regions above or later than regions below. In these older parts susceptibility is often the same in regions where differences in thickness of perisarc are considerable, or it may be different in regions covered by perisarc of equal thickness.

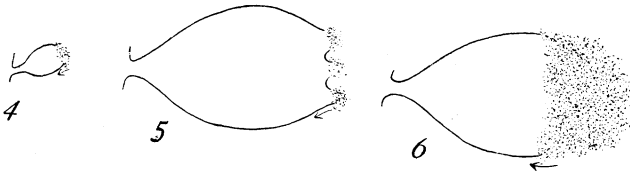
In general the apical region of a growing stem which is to develop into a hydranth is more susceptible than the apical region of a growing stolon of the same colony, but no constant differences have been observed between the older regions of stems and stolons. That these differences between apical ends of stems and stolons represent differences in physiological condition is indicated by the fact, to be more fully discussed in a later paper, that the apical ends of stems can be reversibly transformed into stolons by the action of low concentrations of inhibiting agents.

These data on susceptibility in stems and stolons are in agreement with the facts of observation in that they show the regions of greatest growth activity to be most susceptible to lethal concentrations, and the differences in susceptibility cannot be in-

terpreted wholly in terms of permeability or thickness of perisarc. According to the evidence, the susceptibility relations in the hydroids are very similar to those observed in the multiaxial thalli of algæ (Child, '16a, '16b, '17), and certain other resemblances will be pointed out below.

THE DEATH GRADIENTS IN DEVELOPING MEDUSA BUDS.

The elongated, naked medusa buds of *Pennaria* have constituted the chief material for these observations. In the earlier stages of the bud the disintegration of the ectoderm begins at the free, apical end and progresses basipetally (Fig. 4). In



FIGS. 4-6. Stages of disintegration in Medusa buds of *Pennaria*. Fig. 4. Ectodermal disintegration beginning apically in very early bud. Fig. 5. Later bud stage with disintegration beginning on the tentacular outgrowths. Fig. 6. Later stage of disintegration.

later stages, where the tentacular outgrowths have begun to develop, these disintegrate first of all (Fig. 5), and the process then progresses basipetally over the body, as in earlier stages (Fig. 6). The entoderm disintegrates somewhat later than the ectoderm and shows, at least in earlier stages, a basipetal gradient. The apical region of the developing medusa bud of *Pennaria* represents of course the marginal region of the fully grown medusa, the subumbrellar region not being open to the exterior until an advanced stage of development is reached.

The earlier stages of the gonophores of *Tubularia* likewise show a basipetal susceptibility gradient in the ectoderm and a similar, less distinct gradient in the entoderm. The conditions in the fully developed, free swimming hydromedusae will be considered at another time.

DIFFERENCES IN SUSCEPTIBILITY WITH STAGE OF DEVELOPMENT.

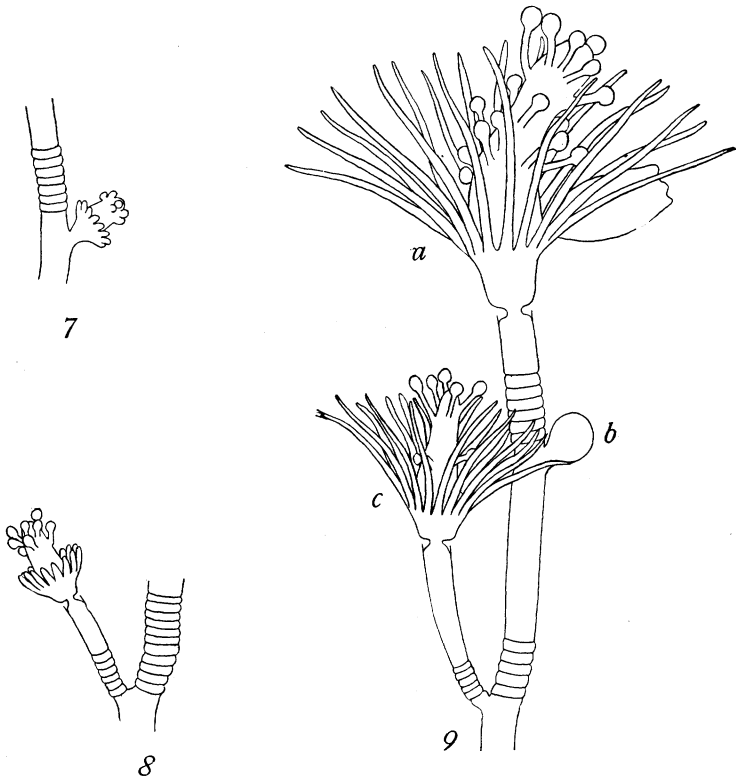
The appearance of motor activity during hydranth development affects susceptibility to some extent as in the case of *Hydra* (Child and Hyman, '19). In general motor activity increases susceptibility, as numerous experiments, not only with *Hydra* and hydroids, but with other forms have shown. The effect of motor activity on susceptibility in *Planaria* has long been used as an experiment in laboratory class work. The earlier stages of hydranth development are non-motile, the later stages motile, e.g., in *Pennaria* the stage of Fig. 7 shows no motor activity, the stage of Fig. 8 is slightly motile and the stages *a* and *c* in Fig. 9 are highly motile. In spite of this difference the early stage of Fig. 7 is distinctly more susceptible than Fig. 9, *a*, but between the stages of Fig. 7, Fig. 8 and Fig. 9, *c* the differences in susceptibility are not very great. In stages after motility is fully developed the susceptibility shows in general a decrease with increasing size of the hydranths on a particular branch or in a particular region of the colony, except that in monopodial forms such as *Pennaria* and *Bougainvillea* certain peculiar conditions exist in the terminal hydranths of the colony and main branches (see p. 114 below).

The effect of motor activity on susceptibility is also evident in the susceptibility of the tentacles, as compared with that of the hydranth body in different developmental stages. In the early stages, e.g., Fig. 7, the tentacles are non-motile and disintegrate at about the same time as the region of the body to which they are attached, but after they develop motility, they are the most susceptible portions of the hydranth, as noted above, and in the forms with two series the proximal tentacles are more susceptible than the distal. In *Pennaria* the proximal tentacles of the advanced stage, Fig. 9, *a*, begin to disintegrate at about the same time or a little later than those of the early stage, Fig. 7, but at this time the body of the early stage is disintegrated down to the level of the proximal tentacles, while the body of stage *a*, Fig. 9 does not begin to disintegrate until later, the actual times depending on concentration of the agent used.

In all other forms examined, *Tubularia*, *Bougainvillea*, the

campanularian, *Obelia*, the earlier stages are in general more susceptible than the later, though some differences depending on motor activity and on position in the colony (pp. 112-117) appear.

The earliest stages of bud development (Fig. 9, *a*, Fig. 10, *g*, are, with rare exceptions, less susceptible than naked early hy-



FIGS. 7-9. *Pennaria tiarella*. Figs. 7 and 8. Early hydranth stages. Fig. 9. Apical region of a stem, showing monopodial form of branching. The hydranth *a* remains apical, the stem below it increases in length and the bud *b* becomes a lateral branch like *c*.

dranths. Since these early stages are enclosed in a thin perisarc they are perhaps not directly comparable as regards susceptibility with later naked stages, but in *Obelia* they are in general less susceptible than later stages such as *b* and *f*, Fig. 10, which are also enclosed in perisarc. On the other hand, they are more susceptible than the stem regions from which they arise. These

facts indicate that real physiological differences exist in the different bud stages. Probably the lower susceptibility of the earliest as compared with later stages means that they have not yet attained their maximum physiological activity as regions of growth. The period of early bud development is certainly a period of acceleration in physiological activity and so of increasing susceptibility like the earlier stages of embryonic development (Child, '15a, Chap. XV.). Later, as differentiation of the hydranth progresses, susceptibility again decreases.

The sensitiveness of these hydroids to laboratory conditions, as well as the close relation between susceptibility and physiological condition is indicated by the fact that colonies kept over night in standing water or even in aquaria with running water very frequently show, either little or no difference or a more or less complete reversal as regards the susceptibility of different stages of development to KNC, *i.e.*, those stages which were originally most susceptible are either no more susceptible or even less susceptible than other stages. A similar reversal appears when the animals are stained for several hours in neutral red and then killed in KNC. Reversals have also been observed in various algæ under the same conditions (Child, '16b, '17). In the hydroids, as in the algæ, it is evident that laboratory conditions or neutral red alter the physiological condition of the earlier, originally more susceptible developmental stages to a greater degree than that of the later stages, and that this alteration decreases the susceptibility of the earlier stages to KNC to such an extent that it is equal to or less than that of later stages. Since susceptibility to KNC shows a close relation to rate of oxidation, it is probable that laboratory conditions and neutral red decrease oxidation in some way and to a greater degree in earlier than in later stages. In other words, this effect of laboratory conditions is itself dependent upon the differences in susceptibility which exist in these organisms. Moreover, this effect and that of neutral red on the one hand, and that of KNC on the other are evidently not additive, as the effect of KNC and lack of oxygen appear to be (Child, '19b).

The susceptibility of the medusa bud, like that of the hydranth, decreases with advancing development in all stages preceding

the development of motor activity, except the very earliest. In *Pennaria* the earliest visible stages show no distinct difference in susceptibility from the hydranth body on which they arise, but somewhat later stages (Fig. 4) are slightly more susceptible than the hydranth body, though distinctly less susceptible than young hydranths of the stage of Fig. 7. Half grown buds are almost always slightly less susceptible than the hydranth body to which they are attached, and the still later stages are much less susceptible than the hydranth and are the least susceptible structures in the colony except the older parts of the stems.

In *Tubularia* the gonophores, which are modified medusæ, show a decrease in susceptibility from a certain early stage on, and the later stages are less susceptible than the hydranth from which they arise. In *Bougainvillea* also, where the medusæ arise singly from special buds instead of from the hydranth body, the differences in susceptibility with stage of development are the same.

The gonozooid of the campanularian species examined is in general less susceptible than the fully developed hydranth, and even the earlier stages of gonozooid development are less susceptible than early hydranth stages, though more susceptible than late hydranths, in spite of the presence of perisarc. The medusa buds on the blastostyle show differences in susceptibility with developmental stage similar to those observed in the medusa bud of *Pennaria*, the advanced stages being much less susceptible than corresponding hydranth stages.

The results with medusa buds of different species are then consistent. The medusa bud represents a reproductive process with a certain amount of dedifferentiation in the earliest stages, and in these stages some increase in susceptibility appears, as compared with the region from which the bud arises, but as differentiation progresses, the susceptibility again decreases. The changes are similar to those in the hydranth, except as the latter are masked by the development of motor activity in relatively early stages. The susceptibility of the medusa bud, however, is in general lower than that of corresponding stages of hydranth development, a fact which agrees well with the greater differentiation of the medusa. In short,

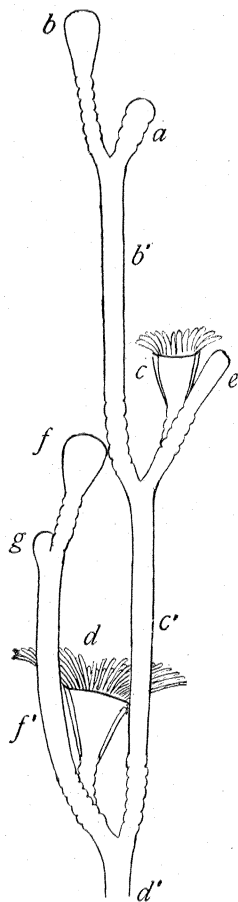


FIG. 10. Apical region of stem of *Obelia borealis* Nutting, to show sympodial method of branching: (a) Latest bud on main axis; arose as a lateral bud from the axis bb' . The axis bb' arose as a lateral bud from the axis cc' , and axis cc' as lateral bud from axis dd' . As the stem of hydranth d elongated a second bud appeared and produced the axis ff' , and from this axis a new axis g is just developing. The stem of hydranth c has also produced a second bud which has given rise to the axis e . In this way the lateral branches develop.

the susceptibility relations indicate that the medusa represents physiologically a more advanced stage of development than the fully developed hydranth and is therefore physiologically older and less susceptible.

COLONY GRADIENTS.

The hydroid colony as a whole represents a more or less orderly integration of the constituent units. The general growth form or arrangement of zooids is more or less definite and characteristic for the species, and this definiteness of form and arrangement is particularly characteristic of those species in which a main stem and lateral branches, with perhaps secondary and tertiary branches can be distinguished. In these respects the hydroid colonies resemble multiaxial plants, and such plants are colonial forms in the same sense as the hydroids, *i.e.*, each growing tip in the plant, like each zooid in the hydroid, represents at least the apical region of a physiological axis, an individual, with more or less complete development of other parts. Moreover, the two general types of relation between the different constituent axes which are characteristic of plants, *viz.*, the monopodial and the sympodial forms of branching, are also found in the hydroids. In *Pennaria* and *Bougainvillea* and various other tubularian hydroids the colony is of the monopodial type, *i.e.*, new axes arise as lateral branches of a persistent main axis (Fig. 9). In *Obelia* and various campanularians examined the colony is sympodial in type. Here each new bud becomes the apical end of the apparent main axis, the former apical zooid being dis-

placed and becoming the tip of a lateral branch (Fig. 10). In these forms what is apparently the main axis is actually made up of a portion of each successive axis formed. In the monopodial *Pennaria* the apical zoöid is, except for injuries or losses, the first zoöid developed and so the oldest in point of time of that stem or branch, while in *Obelia* the apical zoöid is the latest developed and the youngest in point of time, except for the new bud which is soon to take its place.

In both types, however, certain characteristic relations exist between main axes, real or apparent, and lateral branches. In *Pennaria* and the *Obelia* species examined, for example, lateral branches grow more slowly than the main axis, so that the colony developing without injury or crowding shows more or less the general form of a conifer. It has been experimentally determined that in plants of such form very definite physiological relations exist between main and lateral axes, and it has been possible to show for various algæ of these growth forms that the susceptibility relations are likewise very definite in character (Child, '16a, b, '17). The similarity of growth form in plants and hydroids indicates that the physiological relations between the constituent members of the complex must be in certain respects similar and therefore quite independent of the specific differences in plant and animal protoplasm, in other words quantitative in character.

The susceptibility of the hydranths of different regions of the whole colony or of particular stems or branches shows certain characteristic differences which are similar to those found in the algæ and associated in a definite way with the growth relations of the constituent axes. In a well developed colony of *Pennaria* all primary branches, except those latest formed just below the apical end of the main axis, terminate in large hydranths of about the same developmental stage, so far as appearance is concerned, though of course, where losses and regenerations have not occurred, the apical hydranth of the main axis is the oldest in point of time, that of the most basal branch the next oldest and that of each successive branch successively younger. In fresh material, however, the susceptibility of these full grown terminal hydranths decreases in general basipetally in the

colony, *i.e.*, the terminal hydranth of the main axis, although the oldest of all in point of time, and the terminal hydranths of the more apical branches, which are, as regards time, the youngest terminal hydranths, are on the average more susceptible than terminal hydranths of more basal branches. For example, characteristic cases show 60–80 per cent. of the fully developed terminal hydranths of the apical half of a colony disintegrating at a time when 60–80 per cent. of the terminal hydranths of the basal half are still intact. In any well developed primary branch bearing secondary branches, the susceptibility differences in the terminal hydranths of the secondary branches are in general similar to those in the primary terminal hydranths. The terminal hydranth of the primary branch and the hydranths of the more apical secondary branches are on the average more susceptible than those of more basal secondary branches. In short, the colony as a whole and each larger branch shows definite and orderly susceptibility relations among its hydranths, and these relations are similar to those observed in algae with similar growth form. *Bougainvillea* shows similar susceptibility relations, but as might be expected from the less regular form of colony they are less distinct and uniform than in *Pennaria*. It is perhaps necessary to emphasize the point that these are general and average differences and are best determined by comparing percentages of terminal hydranths in process of disintegration at a given time in different regions of the colony, rather than by comparing individual hydranths. The differences are not as great as the differences with stage of development and are clearly brought out only by some statistical method. Even the apical hydranth of the colony does not always disintegrate earlier than any other fully developed hydranth, but it does do so in a large proportion of the cases in fresh material and the relative susceptibility of the other terminal hydranths is likewise not absolutely uniform. In fact this relation in the hydroid is much less distinct and uniform than in many of the algæ.

It is a fact of some interest that in *Pennaria* and *Bougainvillea* the apical hydranth of the colony is the most susceptible or among the most susceptible of the fully developed hydranths, although the oldest in point of time, and that the terminal

hydranths of branches differ in susceptibility as noted above, according to their position in the colony. These differences in physiological condition indicate that for some reason the terminal hydranths of the colony and many branches undergo physiological senescence less rapidly than others. In this connection it may be pointed out that the terminal hydranth is more closely associated than any other with the process of hydranth reproduction, since the new hydranth buds arise a short distance below it (Fig. 9). Moreover, growth and the development of new buds occurs most rapidly in the apical region of the main axis of the colony, and the rate of growth and budding decreases basipetally in the colony as a whole and in each branch. Apparently, as in those plants with similar growth form, there is a relation of dominance and subordination in the colony and in each branch, the apical region being dominant and inhibiting growth and reproduction in other axes to a greater or less degree.

Since new buds arise a short distance below the apical hydranth of any axis (Fig. 9), they probably produce some effect upon the hydranth, either in the way of reorganization or through the demand for nutrition which they create. The nutrition for the bud must be derived largely from the reserves or the newly digested food of the apical hydranth, consequently the nutritive condition of this hydranth may be altered to some degree in the direction of starvation, but when the new hydranth becomes able to obtain food for itself the apical hydranth may recover its former nutritive condition. The effect of starvation in producing regression to a physiologically younger condition in the lower invertebrates has been clearly demonstrated (Child, '14, '15a, Chap. VII, '19a) and it seems probable that alternating changes of this sort associated with the development of new buds are concerned in the retardation of senescence in the apical hydranths. In a monopodial colony such as *Pennaria* the hydranth in which such changes occur most frequently, *i.e.*, the apical hydranth of the colony, will, according to this hypothesis, undergo senescence least rapidly, and the relative rate of senescence in the apical hydranth of any axis will vary in general inversely as rate of growth and frequency of reproduction in that axis. Consequently, since rate of growth and frequency

of reproduction decrease basally in the colony and its branches, the more basal hydranths and regions undergo senescence more rapidly.

The whole complex of physiological relations in the colony represented by the differences in rate of growth, reproduction and susceptibility in different regions results from the order of development. The colony begins as a single axis terminated by a hydranth. When the stem attains a certain length a bud appears at a certain distance below the terminal hydranth and is probably inhibited to some extent by it. As growth of the main stem continues, a second bud arises above the first and now the earlier bud or hydranth is subjected to the inhibiting action of two apical regions, and so on as other buds develop. The same relations arise in each branch as it develops. It follows that in any axial complex the degree of inhibition will increase basipetally.

In *Obelia* and other sympodial forms the situation differs in one respect from that in the monopodial *Pennaria*. The apical hydranth of the colony and of each branch in *Obelia* is the latest developed, the youngest both in point of time and physiologically of the axial complex which it terminates. In well developed colonies of *Obelia*—colonies of *O. borealis* 10–14 cm. in length and very symmetrical in general form are common at Friday Harbor—a general apico-basal gradation in susceptibility of these young apical hydranths or buds of different primary branches appears, those of more apical branches of the colony being somewhat more susceptible than those of more basal branches, like the terminal hydranths of *Pennaria*. Similar differences appear to some degree in the secondary branches of a primary branch. Moreover, the same differences in rate of growth and reproduction as in *Pennaria* appear in different branches from apex to base of the colony or in the secondary branches of any branch, in other words the same relations of dominance and subordination exist in the two growth forms as wholes.

The chief difference between the two forms consists in the fact that in the monopodial *Pennaria* the terminal hydranth maintains its dominance over later buds and therefore remains terminal, while in the sympodial *Obelia* the latest bud becomes

dominant and displaces the former terminal hydranth. In this connection it is of interest to note that the *Obelia* bud grows for some time before it differentiates into a hydranth (Fig. 10). During this period of growth it is the seat of intense physiological activity and shows a high susceptibility, which decreases as the hydranth develops, and it is during this period that it displaces the former apical hydranth. In *Pennaria* the bud differentiates into a hydranth at once with but little growth in length (Fig. 7). That these differences in behavior are associated in some way with the differences in growth form cannot be doubted, and it seems probable that they may be the determining factor. Since dominance in axial relations has been shown to be fundamentally associated with differences in intensity of metabolic activity the intense growth activity of the bud of *Obelia* and similar forms probably enables it to dominate and displace the former apical bud which has completed its period of rapid growth and is undergoing differentiation into a hydranth. In *Pennaria*, on the other hand, where the bud differentiates with very little growth, the intensity of its activity decreases from an early stage and is apparently less at any stage than in the *Obelia* bud, consequently it is unable to alter existing axial relations and becomes a subordinate member of the complex. This interpretation is offered merely as a suggestion, based on the data at hand. Whether it is correct or not can probably be determined only by experimental work such as has been done with plants on control and modification of the relations of dominance and subordination by inhibition or removal of certain apical regions, but such work offers difficulties at present.

The necessity of using freshly collected material for the study of the regional differences in susceptibility, the colony gradients, as well as for other work on susceptibility may once more be emphasized. In colonies kept in the laboratory over night the regional differences have often disappeared or are reversed in the colony or branch.

That there is a real difference in physiological condition in different regions of the colony is indicated by the fact that the medusa buds in *Pennaria* and *Bougainvillea* and the gonozooids in *Obelia* appear first in the most basal regions of the most basal

branches, *i.e.*, the regions where the susceptibility is on the average lower than anywhere else in the colony. From this region the localization of developing medusa buds and gonozooids gradually progresses apically in the colony as a whole and in each branch. Moreover, at least many hydranths of *Pennaria*, after producing medusa buds for a time, degenerate and may be replaced by new ones, but whether such degeneration always follows medusa bud-formation, it is impossible to say. In *Obelia* the hydranths, which in this form have no direct part in the production of medusae, undergo disintegration or resorption, at least very frequently in regions of the colony basal to the level of gonozooid formation. New hydranths may develop in place of the old, so that this loss of hydranths in relation to gonozooid formation may readily escape notice, unless carefully looked for.

Evidently the physiological condition which determines the development of medusa buds and gonozooids is attained in the basal regions of the colony earlier than elsewhere, and gradually progresses apically in the colony, followed by a zone in which at least many of the hydranths die or undergo resorption. This region where medusa buds or gonozooids make their first appearance is the region where rate of growth and frequency of hydranth buds has been least and therefore physiological senescence has progressed most rapidly. In the sympodial colony of *Obelia* these regions are the oldest in time as well as physiologically, while in the monopodial *Pennaria* and *Bougainvillea* they are the oldest in time except the apical hydranths, but physiologically are the oldest of all, apparently because senescence has been retarded in the apical hydranths by the higher frequency of hydranth reproduction. There can be no doubt that the appearance of medusa buds or gonozooids in a colony is associated with the attainment of a certain stage of progressive development and physiological senescence, and the facts presented above, with their interpretation, throw some light on the problem of this first appearance in basal regions and gradual progression apically in the colony.

In various algæ of similar growth form to the hydroids the specialized reproductive organs, *e.g.*, the cystocarps in numerous

species, the stichidia in *Dasya* and related forms, make their first appearance in the basal regions of the thallus and the zone of their development progresses apically, exactly as in the hydroids. Moreover, in the algæ as in the hydroids, the basal regions are the regions of lowest susceptibility and the oldest physiologically (Child, '16 *a*, *b*). That this similarity of localization of specialized reproductive parts, as well as the similarity of growth form in algæ and hydroids is associated with the gradations in physiological condition, of which the susceptibility relations are an indicator, cannot be doubted. And finally, it may be pointed out once more that this similarity in growth form, susceptibility relations and localization of specialized reproductive processes in algæ and many other plants and hydroids must depend upon some physiological factor which is independent of the specific differences in protoplasmic constitution of the different forms and which must therefore be quantitative in character. In short, all the facts force us to recognize the existence of a quantitative gradation in physiological condition, in rate of fundamental metabolism and the associated protoplasmic conditions, as the basis of the axial relations, of which growth form, susceptibility and localization of parts are various expressions.

Concerning the nature of the relation of dominance and subordination which has long been known to exist in the plants and is evidently present in the hydroids also, it need only be said here that the conception of dominance by means of inhibiting, formative or other specific substances accounts neither for the origin nor for the maintenance of these relations. In order that one region may produce substances that inhibit another region, the two regions must already be different, *i.e.*, in order that such chemical relations may exist along a physiological axis, the axis must already be established and certain differences be present. Moreover, if one apical region produces substances which inhibit other apical regions, the one region must be immune to the substances which it produces, while the other regions are not, that is to say differences must already exist between the different apical regions before one can inhibit another without itself being inhibited, and if differences already exist, the assump-

tion of the existence of inhibiting substances becomes wholly unnecessary. And finally, the definite and characteristic spatial relations between different axes in such a complex as the multi-axial plant and the hydroid cannot be accounted for in terms of the production and transportation of inhibiting or other substances. No sort of transportative relation can be conceived which affords an adequate interpretation of the facts. Only on the basis of transmission in protoplasm is it possible to account for both origin and maintenance of the relations which are demonstrated to be present in the organism. Of course after parts have become qualitatively different, more or less specific chemical relations undoubtedly exist between them, but these are results of physiological integration, not its determining conditions. Further discussion is postponed until additional evidence along other lines is presented.

GRADIENTS IN STAINING WITH VITAL DYES.

When neutral red or methylene blue is used in determining differences in susceptibility, it is found that while these dyes enter all cells of the hydranth readily, differences in rate of staining appear in the earlier stages of the process. These differences show the same relations to the axes and to stage of development as the differences in susceptibility. The axial gradients in rate of staining indicate the existence either of differences in permeability to the dyes or of differences in the rate at which they are adsorbed or otherwise taken up by the cell constituents, or perhaps differences of both sorts. That the dyes are held in some way within the cells is evident from the fact that they accumulate there until their concentration is far higher than outside and do not pass out to an appreciable degree when the animals are returned to water. Similar differences in rate of staining with vital dyes, showing in general the same regional relations as the differences in susceptibility, have been noted in many other forms, both plant and animal, but it is evident that in the hydroids, as elsewhere, the differences in susceptibility to the dyes, as measured by time of death, cannot be due alone to the fact that the dyes enter certain cells more rapidly than others. Most of these differences in rate of staining, *e.g.*, those in dif-

ferent parts of the hydranth are slight or only temporarily visible in the earlier stages of staining, but the more extreme differences, such as those between hydranths and stems, remain visible for a longer time, in fact the hydranths may be killed by the stain before the stem is very deeply stained. In general, however, the differences which appear at first tend to become equalized as staining progresses. Apparently the rate of accumulation of the dye decreases most rapidly in those cells which at first stain most rapidly, so that the early differences in rate of staining are obliterated, at least to a large extent, by the action of the dye on the cells.

Nevertheless the differences in susceptibility, as indicated by survival time are very marked in these dyes, even in regions where the staining appears to be uniform in degree. The early differences in rate of staining are evidently not alone responsible for the differences in susceptibility. In fact it is much more probable, as has been suggested elsewhere (Child, '16*b*, Child and Hyman, '19), that the conditions indicated by the differences in staining rate, whether differences in permeability, adsorption or some other factor, are simply one aspect of the differences in fundamental physiological condition which determine differences in susceptibility. The facts of acclimation briefly referred to below, so far as they concern the vital dyes, show even more clearly that the differences in physiological effect are not due to the initial differences in rate of staining, for parts which stain at first more rapidly show a greater capacity for acclimation than less rapidly staining parts.

As death approaches in cells stained with neutral red, a distinct change in the color of the dye toward the acid side occurs, *i.e.*, the cell contents become distinctly acid shortly before disintegration. This change in color of neutral red occurs quite independently of the nature of the killing agent, which may, for example, be neutral red itself, an acid, KNC which is alkaline in solution, etc. Of course as soon as disintegration occurs the color of the neutral red is determined by the hydrogen ion concentration of the entering solution. The increase in acidity preceding death is sufficient to be clearly visible and in elongated axiate parts, *e.g.*, the tentacle or hydranth body, a gradient in

acidity precedes the disintegration gradient. Similar increase in acidity preceding death has been observed in the cells of many algæ (Child, '16 *a*, *b*, '17). In many species of algæ this gradient in acidity is distinct within the length of a single elongated cell, even though the staining may have been quite uniform up to this stage. The axial relations of this increase in acidity are of course the same as those of the disintegrative changes and the color change of the dye may itself be used to a considerable extent as a criterion of susceptibility.

ACCLIMATION.

Presentation of certain data which bear more or less directly upon the problem of acclimation, *e.g.*, the resorption and re-development of hydranths, the transformation of apical regions from hydranth production to stolon production and the reverse, must be deferred to another paper. For the present it may be noted that in low concentrations of various agents, KNC $m/50,000$ – $m/20,000$, HCl $m/2,000$ – $m/1,500$, LiCl $m/50$, ethyl urethane $m/2,000$ – $m/500$, $MgSO_4$ $m/500$, neutral red and methylene blue, indications of differential acclimation appear. In the single hydranth under such conditions the body may die before the tentacles, but in most hydroids the hydranths undergo either disintegration or resorption so rapidly under slightly depressing conditions in the laboratory that the investigation of differential acclimation in the single hydranth is difficult.

Attention has been chiefly directed, however, to the regional differences in acclimation in the colony as a whole, which are very definite and characteristic. In general, the physiologically older hydranth shows less capacity than the younger hydranth or the bud for acclimation to slight inhibitions, consequently, in the course of a few days under such conditions, the older hydranths disintegrate or undergo resorption, while the younger persist or are affected to a less degree or less rapidly. Since the order of arrangement of hydranths of different physiological age along each axis and in the colony as a whole is definite and characteristic for the species (pp. 112–117), the regional differences in acclimation to low concentrations are in general the same as the differences in susceptibility to lethal concentrations, that is,

those zoöids which are most susceptible to the lethal concentrations, show the greatest capacity for acclimation to a certain range of low concentrations.

CONCLUSION AND SUMMARY.

The significant points in the experimental data are; first, that the susceptibility relations are definite, not merely for the single tentacle, hydranth and medusa bud, but for the single axis as a whole and for the axial complexes which result from branching and finally for the whole colony; second that they are similar to the relations observed in other organisms with similar growth forms, *e.g.*, the algæ. In general, the more apical regions of the physiologically younger axes of a complex, which are obviously the regions of most intense metabolism, are most susceptible to the higher concentrations or intensities and also most capable of becoming acclimated to slightly inhibiting conditions. These differences in susceptibility and capacity for acclimation are manifestly associated with quantitative differences in physiological condition, which are fundamental characteristics of physiological axes. The similarity of susceptibility relations in algæ and hydroids of similar growth form, indicates, if it does not demonstrate their independence of the specific differences in algal and hydroid protoplasms and therefore their essentially quantitative character. In spite of the differences in rate of staining with vital dyes, it is evident that differences in susceptibility cannot depend solely upon differences in permeability of limiting surfaces, though such differences in permeability are doubtless associated with the conditions on which susceptibility depends. Regional or axial differences in permeability are themselves dependent upon the fact that the membranes are living and therefore metabolically active, and upon the possibility of quantitative differences in this living condition. Some substances enter cells only as they produce irreversible changes in the living surface, while others enter all cells readily without appreciable injury to the surfaces, yet to both groups of substances the axial and regional differences in susceptibility are in general similar.

These data on susceptibility in the hydroids are in complete

agreement with other data on both animals and plants and constitute another bit of evidence in support of the conclusion that physiological axes are primarily gradients in rate of fundamental dynamic processes and in associated physical and structural conditions in living protoplasm.

The most important facts are summarized as follows:

1. In the hydranths of *Pennaria*, *Tubularia*, *Bougainvillea*, *Obelia*, *Gonothyria* and various other forms, the susceptibility to lethal concentrations of at least many external agents is greatest in the apical region of each tentacle and of the hydranth body and decreases basipetally.

2. In the elongated medusa bud of *Pennaria* the susceptibility decreases basipetally from the apical region.

3. Stems are in general much less susceptible than the more highly specialized structures, though the perisarc may act as a protective covering to some degree. At least in the more apical regions of axes the susceptibility of the stem also decreases basipetally, but in the more basal regions irregularities may appear.

4. Physiologically older zooids or medusa buds are less susceptible than younger, except in certain cases where the development of motility increases susceptibility to such an extent as to overbalance the age differences.

5. Except in the earlier stages the medusa buds of *Pennaria* are less susceptible to lethal concentrations than the hydranth bodies from which they arise, and the gonophores and gonozooids of other forms show the same relations.

6. In the colony as a whole and in each axial complex the average susceptibility of well developed hydranths decreases basipetally, but certain characteristic differences in axial relations exist in the monopodial (*Pennaria*) and the sympodial type (*Obelia*).

7. The medusa buds of *Pennaria* and *Bougainvillea* and the gonozooids of *Obelia*, etc., appear first on those hydranths or in those regions of the colony where the susceptibility to lethal concentrations of agents is least, *i.e.*, in the basal regions of the more basal branches. From these regions the zoë of development of these parts gradually progresses apically as the different levels successively attain the physiological condition which determines their development.

8. The very close relation between susceptibility and physiological condition is indicated by the fact that in various species the characteristic gradients and regional differences in susceptibility often disappear or are reversed after a day or two, or in some cases, after a few hours under laboratory conditions.

9. In general the regions which are more susceptible to lethal concentrations are more capable of acclimation to a certain range of lower concentrations.

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